GENETIC ANALYSES OF ONCORHYNCHUS NERKA 9009300

SHORT DESCRIPTION:
Provide biological and genetic information on O. nerka samples collected throughout the Snake and Columbia River Basins to be used in the overall recovery effort of the Snake River Sockeye Salmon.

SPONSOR/CONTRACTOR: Univ. ID
University of Idaho
Dr. Ernest Brannon, Director, Aquaculture Research Institute
Aquaculture Research Institute, University of Idaho, Moscow, ID 83844-2260
208/885-5830 Aqua@uidaho.edu

SUB-CONTRACTORS:
N/A There will be no subcontractors to the University of Idaho under this contract.

GOALS

GENERAL:
Supports a healthy Columbia basin, Maintains biological diversity, Maintains genetic integrity

ANADROMOUS FISH:
Research, M&E

NPPC PROGRAM MEASURE:
7.5A.1

RELATION TO MEASURE:
This project's overall objectives and long term goals have not changed. This project relates to the NPPC program measure as previously noted.

BIOLOGICAL OPINION ID:
NMFS-Hydrosystems Operations Biological Opinion and Endangered Species Act Requirements

OTHER PLANNING DOCUMENTS:
Snake River Salmon Recovery Plan

TARGET STOCK	LIFE STAGE	MGMT CODE (see below)
ColumbiaBasin Oncorhynchus nerka	All	S
Snake River sockeye	All	L

AFFECTED STOCK	BENEFIT OR DETRIMENT
Columbia Basin Oncorhynchus nerka	Beneficial

BACKGROUND

STREAM AREA AFFECTED

Stream name:
Salmon River

Subbasin:
Snake River

Stream miles affected:
N/A Redfish Lake is affected.

Hydro project mitigated:
N/A No particular project is identified.

Project is an office site only

Habitat types:
N/A The on going work involves the genetic analysis of tissue samples taken from fish, throughout the Columbia Basin.

HISTORY:

Activities have included rearing Redfish Lake kokanee to examine inter-year temperature unit variability for egg incubation and behaviorally for downstream migration volitionally out of circular tanks. Primarily work has focused on defining a technique and regions of DNA base sequences useful for separating the life history forms of O. nerka in Redfish Lake. Costs have included renovation of experimental wet laboratory facilities, equipment for genetic laboratory, salaries and supplies. Benefits are that regions useful for diagnostic purposes have been identified for the anadromous versus non-anadromous components of Redfish Lake O. nerka and a wet lab that can be used for small scale incubation and rearing projects.

PROJECT REPORTS AND PAPERS:

Genetic Analysis of Oncorhynchus Nerka - Annual Progress Reports FY 1991, FY 1992, Genetic Analysis of O. Nerka: Life History and Genetic Analysis of Redfish Lake O. Nerka, Completion Report FY 1993-1994. Monthy Progress Reports 1991 to 12/1996 and Stanley Basin Sockeye Technical Oversight Committee Meeting Notes 1993-12/1996.

ADAPTIVE MANAGEMENT IMPLICATIONS:

Our objectives are directed at resolving the origins and phylogeographic relationships of existing O. nerka stocks in the Columbia Basin. Additionally, we wish to determine what level of gene flow exists between populations and what genetic contributions are made by anadromous and resident populations to reciprocal migratory forms. The results of this research will influence management decisions regarding the uniqueness of various sockeye stocks and their designation as evolutionary significant units (ESU). This work has far reaching implications regarding the Endangered Species Act as well as future conservation efforts for non-endangered populations of trout and salmon.

PURPOSE AND METHODS

SPECIFIC MEASUREABLE OBJECTIVES:

Comprehensively identify the genetic structure of Redfish Lake O. nerka outmigrant-originating populations. Provide long term information about the genetic identity of returning anadromous sockeye as this run is restored. Define the relatedness of populations of O. nerka in Redfish, Stanley, and Snake River lakes and the Columbia Basin lakes. Provide information to monitor the change or loss of genetic biodiversity among O. nerka populations throughout the Columbia Basin and in particular, Redfish Lake, Idaho. As stated above, these objectives are directed at resolving the origins and genetic relationships of existing stocks of O. nerka in the Columbia Basin with the goals of determining what level of divergence exists between populations and what genetic contributions are made by anadromous and resident populations to reciprocal migratory forms.

CRITICAL UNCERTAINTIES:

Please refer to the section B. (below) for a discussion on the use of larger sample sizes. It should be noted that an assumption has been made regarding the long term survival of Snake River sockeye and their genetic adaptability. We have assumed, until proven otherwise, that sufficient genetic variation exists within the anadromous, beach spawning, and/or sympatric kokanee population to address that organism's natural environmental challenges to survival. The level of genetic variation needed, changes with the organism under study and with the stability of its environment. Some organisms thrive under conditions of little genetic diversity, but others do not. Consequently, their risk of extinction can be very high. At this point, the level of genetic variation needed in the Snake River sockeye population for good probability of long term survival is unknown. Unfortunately, it is also uncertain whether a sufficient level of genetic variation still exists. The genetic analyses undertaken in this project will help to facilitate an answer to both those questions.

BIOLOGICAL NEED:

The populations of sockeye salmon present in the Snake river have been considered unique in that their genetics and life history patterns are divergent, to a sufficient degree, from other stocks of sockeye salmon. Under the tenets of the U. S. Endangered Species Act, these fish are now protected. However, the recovery of these fish populations to sustainable levels requires genetic monitoring because long term survival will be influenced not only by the restoration and preservation of their habitat but, also by their ability to genetically contend with their historically stochastic environment. This project is essential to any recovery strategy concerned with the maintenance of genetic variation and diversity within this species.

HYPOTHESIS TO BE TESTED:

The ongoing analysis and proposed future work will be used to test the following hypotheses:

1) Anadromous, Redfish Lake sockeye salmon are a distinct population of O. nerka and represent an evolutionarily significant unit (ESU) recognized under the Endangered Species Act and are following an independent evolutionary trajectory as evidenced by reduced gene flow relative to a sympatric kokanee population.
2) Beach spawning, resident sockeye are an intermediate form between anadromous sockeye and kokanee but, most closely resemble anadromous sockeye in a taxonomic sense leading to their inclusion in the Redfish Lake ESU.
3) The sympatric kokanee population present in Redfish Lake is not significantly contributing to the numbers of outmigrating anadromous sockeye and thus the Redfish Lake ESU.
Populations of O. nerka within the Columbia and Snake River basins form genetically distinct stocks due to their high degree of philopatry and thus may give rise to additional situations where a sockeye populations become reduced in number and have to be considered for protection under the ESA.

ALTERNATIVE APPROACHES:
Alternative approaches to accomplishing this projects objectives arise in the form of alternative approaches to the gathering and analysis of genetic data. The particular methods used in this project have been selected based upon their informativeness, reproducibility, cost effectiveness, and productivity.

JUSTIFICATION FOR PLANNING:
N/A This project involves applied research to provide information to fisheries managers.

METHODS:
Two types of DNA analysis are currently employed. The first involves the development and use of polymorphic mitochondrial gene loci. The second is the development and use of nuclear DNA markers resulting from polymorphisms within mini- and microsatellite loci.
For mitochondrial DNA (mtDNA) restriction fragment length polymorphism (RFLP) analysis, isolated mtDNA is amplified using the polymerase chain reaction (PCR) and primers specific for four separate gene regions; ND1, ND2, ND5/6, and Cytochrome b. The amplified regions of DNA are digested using restriction endonucleases. The resulting banding patterns are then evaluated using numerical taxonomy programs such as NTSYS-pc or clustering programs such as REAP.
For nuclear genome analysis, total genomic DNA is isolated from non-destructively collected tissue samples and digested with one of several restriction endonucleases. The digested DNA is electrophoresed in an agarose gel then transferred via Southern blotting to a nitrocellulose or nylon membrane. The transferred DNA is fixed to the membrane and subsequently hybridized with a non-radioactively labeled fragment of DNA used as a marker. The resulting banding patterns, documented using x-ray film, are then scored and analyzed using various phylogenetic inference and numerical taxonomy programs.

PLANNED ACTIVITIES

SCHEDULE:

Planning Phase **Start** 1990 **End** 1994 **Subcontractor** Washington State University

Task Develop techniques used in the analysis of mitochondrial DNA-RFLPs in O. nerka. Develop additional DNA markers using nuclear DNA to differentiate Redfish Lake populations of sockeye salmon and kokanee. Creation and set-up of molecular and wet laboratory facilities. Collection and archiving of sample tissue for analysis.

Implementation Phase **Start** 1993 **End** 1999 **Subcontractor** Washington State University

Task Analyze tissue samples from at least 32 individuals per population using mtDNA-RFLP analysis and previously developed approaches. Continue to describe haplotype differences and substructure among early and late spawning O. nerka populations. Test volitional outmigration from tanks, salt water tolerance etc. from reciprocal crosses of kokanee/sockeye. Develop additional DNA markers using nuclear DNA to differentiate Redfish Lake populations of sockeye salmon and kokanee. Verify the presence of pseudogenes within the nuclear genome and develop them as potential markers to eliminate any bias in conclusions regarding genetic relatedness and variation based on maternally inherited (mtDNA) polymorphisms alone.

O&M Phase **Start** 1997 **End** 2002 **Subcontractor** none

Task 1) Continue DNA analysis on samples of the outmigrant assemblages to assess contributions from kokanee on subsequent year classes. 2) Continue DNA analysis on samples of progeny from the captive broodstock and returning adults so as to characterize the nature of successful outmigrant contributions. Expand the existing data base by obtaining 60 individual samples for each population. Additional outmigrant samples will be examined to further investigate the possible loss of haplotype diversity in different brood years. Additional approaches to nuclear DNA analysis will be investigated for potential use.

PROJECT COMPLETION DATE:

Unknown. Monitoring will be essential until the goals in the ESU recovery plan are reached.

CONSTRAINTS OR FACTORS THAT MAY CAUSE SCHEDULE OR BUDGET CHANGES:

There is no known risk associated with non-destructive sampling and genetic analysis of O. nerka populations.

OUTCOMES, MONITORING AND EVALUATION

SUMMARY OF EXPECTED OUTCOMES

Expected performance of target population or quality change in land area affected:

This project will result in a comprehensive data base and genetic profile from which the immediate and long term genetic risks to Snake River sockeye as well as other populations or stocks of sockeye within the Columbia River Basin can be addressed.

Present utilization and convservation potential of target population or area:

The present target population is listed as a federally endangered species. Several of the other O. nerka stocks examined in this study are in decline and may be potential candidates for future listing.

Assumed historic status of utilization and conservation potential:

Unknown

Long term expected utilization and conservation potential for target population or habitat:

Recovery of the target population to sustainable levels as listed in the recovery plan for Snake River sockeye salmon.

Contribution toward long-term goal:

The project provides genetic information to fisheries and resource managers to aid in the restoration and recovery of sockeye and kokanee populations in the Columbia River Basin.

Indirect biological or environmental changes:

The genetic information provided may influence any number of management or resource use decisions concerning sockeye salmon and the maintenance of biodiversity in general.

Physical products:

Currently the Aquaculture Research Institute at the University of Idaho has collected, under the auspices of this project, over 3400 tissue samples of O. nerka comprising 32 separate populations throughout the Pacific Northwest and British Columbia.

Environmental attributes affected by the project:

N/A This project does not directly affect any environmental attributes.

Changes assumed or expected for affected environmental attributes:

N/A

Measure of attribute changes:
N/A

Assessment of effects on project outcomes of critical uncertainty:
The critical uncertainties of this project will be most effectively assessed by the outcome, good or bad, of the recovery plan for endangered Snake River sockeye salmon.

Information products:
Provides biological and genetic information on O. nerka samples collected throughout the Snake and Columbia River Basins to be used in the overall recovery effort of the Snake River Sockeye Salmon.

Coordination outcomes:
Isolation of a single locus probe which could distinguish the "a" allele seen primarily in Redfish Lake kokanee. Behaviorally, Fishhook Creek kokanee do not appear to migrate downstream in significant numbers under experimental conditions.
Characterization of outmigrant individuals from Redfish Lake based on the single locus probe. Evolutionary relationship structure based on mitochondrial DNA. The mtDNA information appears to resemble the information collected from the nuclear single locus probe suggesting that the mtDNA information accurately reflects gene flow among the subgroups.
Three forms of O. nerka exist in Redfish Lake: anadromous sockeye, "resident beach spawning," and resident kokanee. Anadromous sockeye and "residual" sockeye spawn on the historical beach spawning site in October and November, respectively, while the resident kokanee spawn in Fishhook Creek during August and September.
Life history characteristics of the three forms were assessed with some differences in development rate of eggs and number of gill rakers counts.
DNA analysis included assessment of other O. nerka stocks in the Salmon/Snake River system, the Upper Columbia River and outside the Columbia River system. Development of DNA nuclear markers or probes is still underway that might readily segregate the three forms. Preliminary results indicate three forms closely related, but may be sufficiently different to be considered three separate stocks.
Development of the single locus probe made it possible to isolate three alleles (A, B, and C) in Redfish Lake. The A allele is absent in sockeye and beach spawners, but present in kokanee. Possible conclusion is that kokanee population is not contributing many outmigrants.
Mitochondrial DNA data have defined all outmigrants examined fall into 4 of 16 possible haplotypes. Continue to determine whether or not kokanee introductions into Stanley Basin Lakes were successful.
Screened outmigrants from Redfish Lake. Data was used to evaluate crosses made with gametes obtained from the captive broodstock program. This allowed genotypes AC, AB, and AA fish from being part of the broodstock. These fish are excluded because they have the A allele which indicates kokanee origin.

MONITORING APPROACH

Provisions to monitor population status or habitat quality:
As previously stated, the target stock is a federally endangered species. Thus, monitoring of the population status falls under the direction of NMFS and Idaho Fish and Game.

Data analysis and evaluation:
Genetic Data from this project will be analyzed using several commomly used statistical programs for this purpose. They include, NTSYS, PHYLIP, PAUP, Sigma STAT, DNAsize, Sigma Scan and several others. The outcomes will be evaluated by project staff as well as members of the Technical Oversight Committee and NMFS.

Information feed back to management decisions:
Through Technical Reports and Technical Oversight Committee meetings

Critical uncertainties affecting project's outcomes:
This project has already undertaken "broader scale" research in order to minimize the critical uncertainties associated with the genetic aspects of recovery of Snake River sockeye salmon.

EVALUATION

The overall performance of the project can be assessed by querying the recipients of this project's information.

Incorporating new information regarding uncertainties:

New information regarding uncertainties of the project will be addressed to the members of the Technical Oversight Committee by members of this project. Any resolutions or suggestions will be evaluated by the TOC and subsequently incorporated into the project's objectives.

Increasing public awareness of F&W activities:

This project does not involve nor, does it contain funding for public relations activities. However, this project is funded at a major land-grant university within the region at a research center that is substantially involved in extention and outreach services. These conditions predicate a certain degree of "visibility" for BPA involvement and concern for the region's efforts to protect, mitigate, and enhance fish and wildlife. Public awareness is also increased by participation of project staff in regional and national scientific meetings and publication in both peer reviewed and non-peer reviewed literature.

RELATIONSHIPS

RELATED BPA PROJECT	RELATIONSHIP
9009300 Genetic Analyses of Oncorhynchus Nerka	provides DNA analysis of O. nerka for this project
9204000 Redfish Lake Sockeye Captive Broodstock Program	
9009300 Genetic Analyses of Oncorhynchus Nerka	provides DNA analysis of O. nerka for this project, especially the captive broodstock spawning matrices
9107200 Idaho Department of Fish and Game Sockeye Salmon Captive Broodstock Program	
9009300 Genetic Analyses of Oncorhynchus Nerka	provides DNA analysis of O. nerka for this project
9107100 Snake River Sockeye Salmon Habitat and Limnological Research (esa)	

RELATED NON-BPA PROJECT	RELATIONSHIP
Salmonid and Freshwater Fish Laboratory/EPSCoR Program NSF	This 2yr. grant provides funding for the formation of a laboratory which will conduct research on endangered fish populations.

OPPORTUNITIES FOR COOPERATION:

Since the Snake River sockeye listed under the endangered Species Act are anadromous, their protection and recovery fall under the jurisdiction of the National Marine Fisheries Service. The broodstock program is conducted by the National Marine Fisheries Service (program # 92040) and the Idaho Department of Fish and Game (program # 91072). Our objectives are commensurate with the responsibilities and objectives of the fore mentioned agencies as well as those of the Sho-Ban Tribe (program # 91071). Thus far this has led to a successful, cooperative, interdisciplinary effort toward the conservation of this endangered species.

COSTS AND FTE

1997 Planned: $134,000

FUTURE FUNDING NEEDS:

FY	$ NEED	% PLAN	% IMPLEMENT	% O AND M
1998	$130,000			
1999	$120,000			
2000	$120,000			
2001	$120,000			
2002	$120,000			

PAST OBLIGATIONS (incl. 1997 if done):

FY	OBLIGATED
1990	$36,858
1991	$596,751
1992	$124,741
1994	$246,586
1995	$223,396
1996	$125,000

1997	$139,994
TOTAL:	$1,493,326

Note: Data are past obligations, or amounts committed by year, not amounts billed. Does not include data for related projects.

FY	OTHER FUNDING SOURCE	AMOUNT	IN-KIND VALUE
1998	EPSCoR/NSF (only through June 1998)	$14,500	

OTHER NON-FINANCIAL SUPPORTERS:
None

LONGER TERM COSTS: Unknown
Operation and Maintenance cost will vary considerably with respect to the future progress of the recovery plan for the target species.

1997 OVERHEAD PERCENT: 44.2%

HOW DOES PERCENTAGE APPLY TO DIRECT COSTS:
All direct costs less capital expenditures.